

# UC Irvine

## UC Irvine Previously Published Works

### Title

A regional increase in the number of hippocampal GABAergic neurons and terminals in the seizure-sensitive gerbil.

### Permalink

<https://escholarship.org/uc/item/69x943hs>

### Journal

Brain research, 340(2)

### ISSN

0006-8993

### Authors

Peterson, GM  
Ribak, CE  
Oertel, WH

### Publication Date

1985-08-01

### DOI

10.1016/0006-8993(85)90937-0

### Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

## A regional increase in the number of hippocampal GABAergic neurons and terminals in the seizure-sensitive gerbil

GARY M. PETERSON<sup>1</sup>, CHARLES E. RIBAK<sup>1</sup> and WOLFGANG H. OERTEL<sup>2</sup>

<sup>1</sup>Department of Anatomy, University of California at Irvine, Irvine, CA 92717 (U.S.A.) and

<sup>2</sup>Neurologische Klinik, Technische Universität München, D-8000 München (F.R.G.)

(Accepted March 26th, 1985)

**Key words:** hippocampus — dentate gyrus —  $\gamma$ -aminobutyric acid (GABA) — glutamic acid decarboxylase (GAD) — epilepsy — genetic model

Inhibitory  $\gamma$ -aminobutyric acidergic (GABAergic) neurons were identified in the dentate gyrus of seizure-sensitive (SS) and seizure-resistant (SR) gerbils by immunocytochemical localization of glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA. Increases in both the number of GAD+ somata and terminals were found in the dentate gyrus of the SS brains compared to the SR. The magnitude of the increase was positively correlated with the recorded seizure intensity. The increased number of GABAergic neurons in the dentate gyrus of SS gerbils could result in disinhibition of the granule cells, thereby allowing propagation of epileptiform activity through the hippocampus.

Alterations in the cortical  $\gamma$ -aminobutyric acidergic (GABAergic) inhibitory system have been demonstrated in animal models of focal epilepsy where a gliotic scar is associated with the epileptic focus. Results from immunocytochemical<sup>27</sup>, electron-microscopic<sup>25,26</sup> and biochemical<sup>6,15</sup> studies strongly indicate that a selective reduction of GABA-mediated inhibition in epileptic foci is responsible for the hyperexcitability of neurons in this region. The intent of the present study is to determine if defects in the GABAergic system also exist in a genetic model of epilepsy.

The Mongolian gerbil (*Meriones unguiculatus*) is an excellent model of epilepsy because the animals exhibit spontaneous seizures which are induced simply by placing the animal in a novel environment<sup>16,17,18</sup>. The seizure intensity is consistent over many testings and therefore it is possible to correlate a known history of seizure intensity with morphological observations. The animals have been bred phenotypically over 10–15 generations to produce two strains, one which is highly seizure sensitive (SS) and one which is seizure resistant (SR). Since seizure ac-

tivity is not exhibited in the SS animals prior to about 50 days of age<sup>17</sup>, it is possible to examine a seizure predisposed (SP) brain prior to the onset of seizures to determine if a defect in the adult is present prior to seizure activity in the young gerbil. Therefore, both the SR and, in a sense, the SP animals provide important controls.

A recent report by Paul et al.<sup>23</sup> demonstrated a loss of spines on hippocampal pyramidal cell dendrites in region CA3 of SS gerbils. Also, the mossy tufts, the en passant terminals of the granule cell axons, showed a greater proportion of total tuft area occupied by vesicles in the same SS animals. These data, together with results from other studies that implicate hippocampal involvement in human epilepsy<sup>9,12,24</sup> led us to investigate this brain region in SR, SS and SP brains with electron microscopy and immunocytochemical methods for glutamic acid decarboxylase (GAD), the enzymatic marker for GABA. The GAD antiserum used in this study was prepared by Oertel et al.<sup>20,21</sup> and offers the advantage of staining both neuronal somata and their terminals without the use of colchicine.

*Correspondence:* G. M. Peterson, Department of Anatomy, University of California at Irvine, Irvine, CA 92717, U.S.A.

Our studies concentrated on the hippocampal formation and, in particular, the dentate gyrus. The normal rat dentate gyrus contains several types of GAD+ cells<sup>29,30</sup>, the most numerous and heterogeneous population being in the hilar region. Seress and Ribak<sup>30</sup> have characterized 4 distinct types of GAD+ basket cells within or immediately subjacent to the stratum granulosum: the pyramidal basket, large fusiform, horizontal basket and inverted fusiform cells. All of these cell types give rise to a pericellular axonal plexus that contacts granule cells<sup>28</sup>. The somata of the basket cells are substantially larger than the somata of the granule cells which do not stain for GAD<sup>29,30</sup>. The stratum moleculare contains relatively few GAD+ somata, these being mainly of a multipolar configuration. GAD+ terminals are most dense within the stratum granulosum where they are closely associated with the granule cell somata<sup>7,11,29</sup>.

Gerbils were tested once each week for seizure intensity as described by Paul et al.<sup>23</sup>. Age-matched SS and SR brains were prepared for immunocytochemistry using an indirect, double-bridged, avidin-biotin method<sup>13,22</sup> (Vectastain, Vector Laboratories). When possible SS, SR and SP brains were processed simultaneously. In addition, SS and SR brains were processed for electron microscopy<sup>26</sup>.

Data analysis involved counting and measuring the size of GAD+ neuronal somata in the dentate gyrus from both cerebral hemispheres. Every fifth section throughout the septotemporal axis was used and 5 subregions were analyzed for somata: the hilar region<sup>1</sup>, the infra- and suprapyramidal blades of stratum granulosum and the infra- and suprapyramidal blades of the stratum moleculare. For purposes of converting cell number to cell density, sections were projected and traced onto a Houston Instruments digitizing tablet supplied with the Bioquant morphometric analysis system (R and M Biometrics) and areas of the two blades of the strata granulosum and moleculare were measured<sup>14</sup>. Cell density was thus defined as number of GAD+ neurons per unit area of tissue and provided a standardized measure for comparison between and within brains. In addition, GAD+ terminal density was estimated by counting the labeled puncta in specific areas of the strata granulosum and moleculare in both infra- and suprapyramidal blades. These puncta have been shown to be the light-microscopic equivalents of GAD+ presyn-

aptic terminals<sup>21,27</sup>. A total of 6 SS, 3 SR and 3 SP brains were examined.

The size and distribution of GAD+ neurons and terminals in the SR dentate gyrus was similar to that in the rat<sup>7,29,30</sup>. Thus, the somata of GAD+ basket cells in the SR stratum granulosum were usually spaced 80–140  $\mu$ m apart and were only rarely found

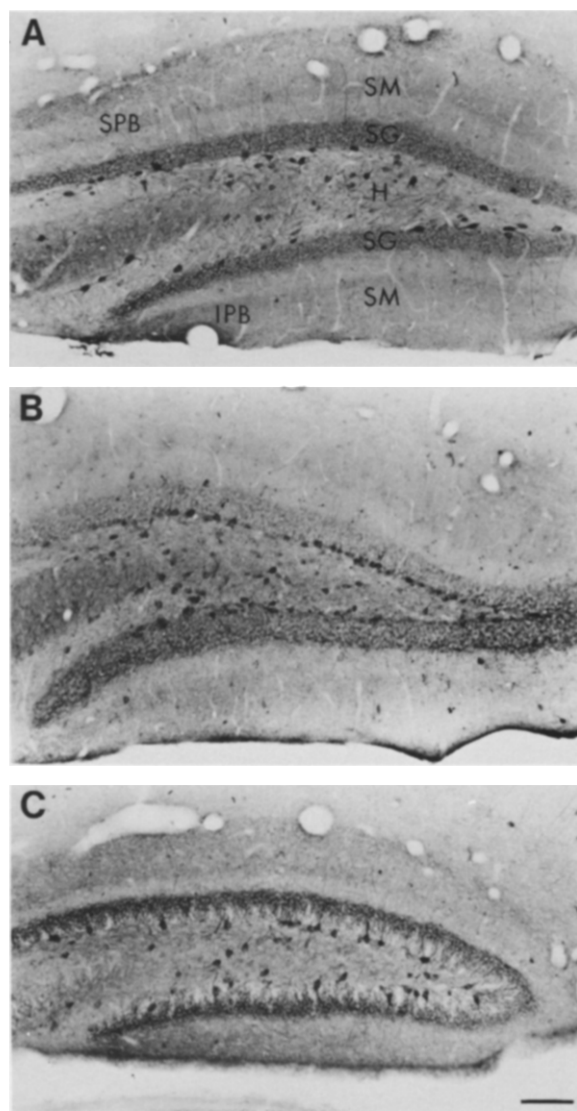


Fig. 1. Coronal sections that were incubated in GAD antiserum show the somal distribution of GABAergic neurons in the gerbil dentate gyrus. GAD+ pyramidal basket cells in SR (A) are found at the border between the stratum granulosum (SG) and hilus (H) in both the supra- (SPB) and infrapyramidal (IPB) blades. Both SS (B) and SP (C) dentate gyri display more GAD+ neurons at this location than the SR. Counts obtained from the hilus and stratum moleculare (SM) also showed increases in the SS brains (see text). Scale bar, 100  $\mu$ m.

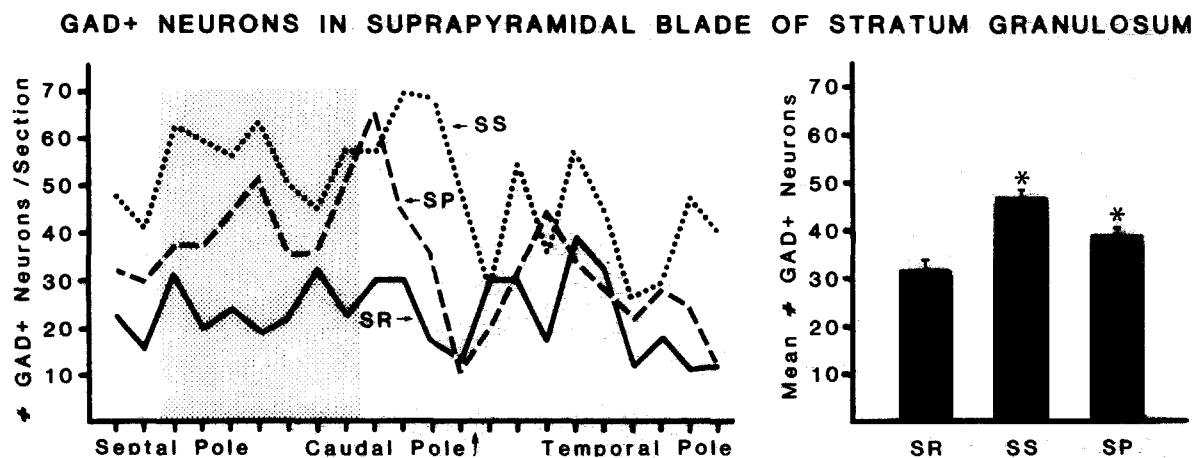
in close proximity to one another. In contrast, GAD+ somata in the SS dentate gyrus were more numerous and often formed groups of 3 or 4 where some appeared to contact each other. In addition, the average size of the GAD+ somata in the SS was 30% smaller than in SR brains ( $\bar{x}_{SS} = 112.6 \mu\text{m}^2$ ,  $\bar{x}_{SR} = 144.1 \mu\text{m}^2$ ,  $P < 0.01$ , Student's *t*-test).

The most striking difference between the SR and SS dentate gyri was the number of GAD+ somata, especially those contained within or having processes passing through the stratum granulosum (Figs. 1 and 2). For example, there were nearly twice as many cells in the suprapyramidal blade of the SS brains as compared to the corresponding region of SR brains. The difference was most substantial and consistent in the septal half of the dentate gyrus (Fig. 2). In contrast, the number of GAD+ cells in the infrapyramidal blade of SS brains was approximately the same as that in the infrapyramidal blade of the SR brains. To determine whether a relationship exists between number of GAD+ cells and seizure intensity all adult animals were included in a regression analysis that showed that the number of GAD+ cells in the stratum granulosum of the suprapyramidal blade was positively correlated with the recorded seizure intensity ( $r = 0.725$ ). The stratum moleculare also displayed more GAD+ neurons in SS brains as com-

pared to SR brains and, again, the difference was most marked in the suprapyramidal blade. In addition to cells in strata granulosum and moleculare, GAD+ neurons were counted in the hilar region. The number of these cells in the SS brains were found to be approx. 20% greater than in the SR brains. Thus, the SS brains displayed more GAD+ somata than the SR brains in all regions of the dentate gyrus.

To more accurately compare the cell counts of GAD+ somata between brains, cell number data from strata granulosum and moleculare were standardized by determining cell density (number of GAD+ somata/mm<sup>2</sup>). These cell density measurements were consistent with the cell count data.

The number of GAD+ puncta within the SR dentate gyrus was similar to that reported in the rat where the outer third of stratum moleculare was moderately dense and the density of these terminal structures in stratum granulosum was uniform between the infra- and suprapyramidal blades. In contrast, the number of GAD+ terminals in the SS dentate gyrus was considerably greater in the infrapyramidal blade of stratum granulosum than in the suprapyramidal blade. The ratio of terminal density of infrapyramidal blade to suprapyramidal blade was nearly 3:1 in the SS whereas in the SR the ratio was approximately 1:1. The terminal density in both



blades of the SS dentate gyrus was significantly greater than in the SR ( $P < 0.01$ ; Student's *t*-test) and the terminals appeared to be both larger and more densely stained in the SS brains, especially in the infrapyramidal blade. No differences were observed in terminal density within the stratum moleculare.

In conjunction with our light-microscopic analysis, we examined the ultrastructural appearance of the mossy tufts, the large axon terminals of the granule cells. Since the light-microscopic data indicated a substantial difference in the number and connections of inhibitory neurons within the dentate gyrus between SR and SS brains, this might be reflected in the activity and, perhaps, in the axonal morphology of the projection cells. The SR brains had mossy tufts that resembled those described in other rodent species<sup>2,3,10,19</sup>. In contrast, many mossy tufts in the SS

brains showed a depletion of synaptic vesicles and an increase in the number of cisternae of agranular reticulum. Also, the mitochondria in these tufts were located close to active zones (Fig. 3). The plasma membrane of SS mossy tufts was highly infolded with some of these infoldings connected to the cisternae. The observed decrease in the number of vesicles in these tufts and the increased number of smooth cisternae are indicative of a high rate of synaptic activity<sup>19</sup>.

The results of the GAD+ cell count data indicate that virtually all regions of the SS dentate gyrus display more GABAergic neurons than that found in the SR. These results appear to contradict the current notion that epileptic activity arises from a selective decrease in GABAergic cells and terminals<sup>6,15,25,26,27</sup>. However, these data were obtained

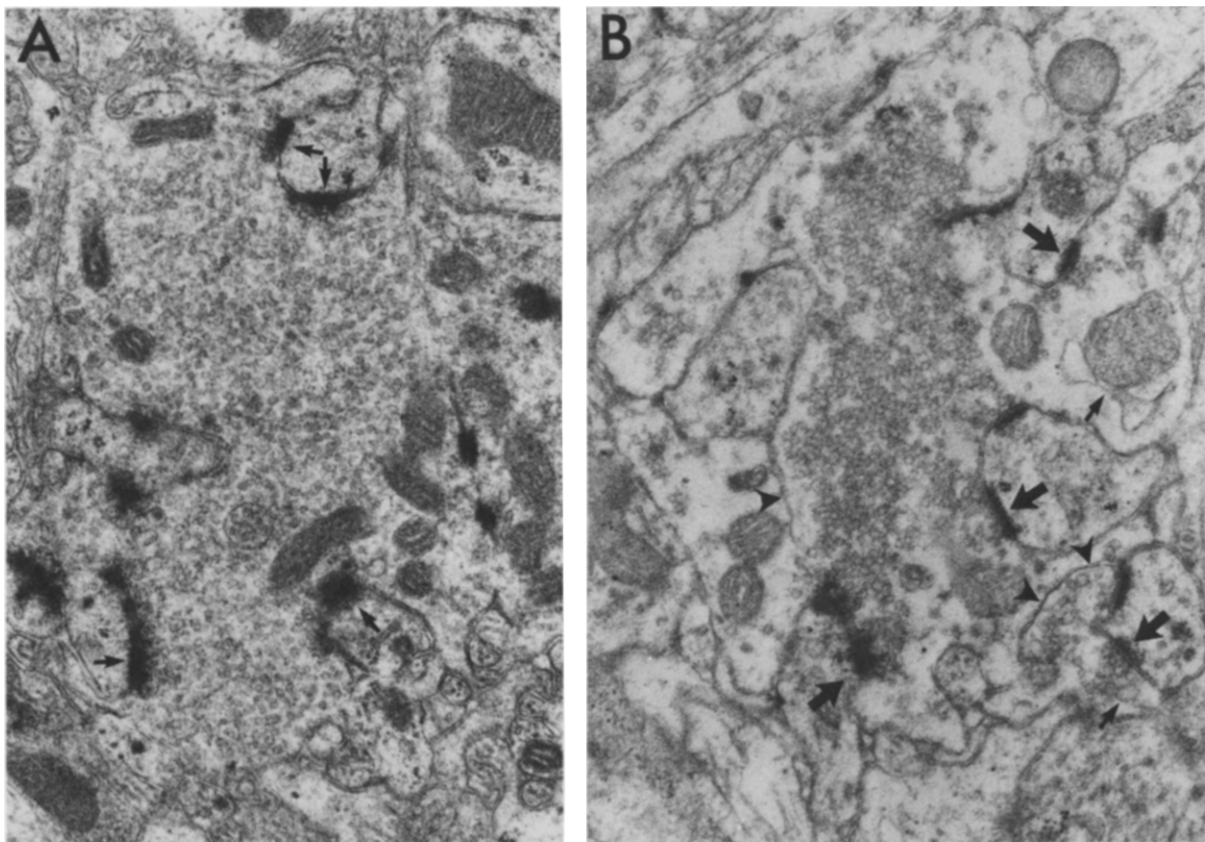


Fig. 3. A: electron micrograph of a mossy tuft from an SR brain. Round synaptic vesicles fill most of this terminal that forms typical asymmetric axospinous synapses (arrows). 21,000 $\times$ . B: a mossy tuft from an SS brain. This terminal displays a depletion of synaptic vesicles, membrane infoldings derived from active sites (arrowheads) and cisternae of agranular reticulum (arrows). These features are typical for 'active' terminals. Asymmetric axospinous synapses (large arrows) are also formed by SS mossy tufts. 24,000 $\times$ .

from models of focal epilepsy whereas our data apply to a genetic model of epilepsy that lacks a specific seizure focus<sup>16</sup>.

The possibility that the increase in the number of GAD+ neurons and terminals is a compensatory response to increased excitatory input is not likely. The number of GAD+ cells in the SP brains, which are genetically predisposed to seizure but have not had seizures, is greater than the number found in SR brains. Thus, the data indicate that the increased number of GAD+ cells in the dentate gyrus is not a result of actual seizure activity but is genetically programmed.

Many of the GAD+ neurons in the dentate gyrus have been shown to be the basket cells<sup>29,30</sup> which provide feedback inhibition to the granule cells<sup>4,5,8</sup>. In the SS brains we have observed an increase in the number of GAD+ terminals within the stratum gran-

ulosum. It is possible that many of these extra terminals form synapses on other basket cells, thereby disinhibiting the granule cells. The active appearance of many SS mossy tufts suggests that granule cells in the dentate gyrus are more active, perhaps due to disinhibition. To test this disinhibition hypothesis for genetic models of epilepsy we plan to examine GAD+ terminals in electron-microscopic preparations to determine if they form more synapses with GAD+ basket cell somata in the dentate gyrus of SS compared to SR gerbils.

Supported by NIH Grant NS 15669 and a Klingenstein Fellowship (C.E.R.). We thank M. Brundage, Y. Jhurani, K. Andersen and S. Khan for technical assistance, Drs. L. Paul and A. Scheibel for the gerbil colony, Dr. C. Gall for reviewing the manuscript and N. Sepion for secretarial assistance.

- 1 Amaral, D. G., A Golgi study of cell types in the hilar region of the hippocampus in the rat, *J. comp. Neurol.*, 182 (1978) 851-914.
- 2 Amaral, D. G., Synaptic extensions from the mossy fibers of the fascia dentata, *Anat. Embryol.*, 155 (1979) 241-251.
- 3 Amaral, D. G. and Dent, J., Development of the mossy fibers of the dentate gyrus. I. A light and electron microscopic study of the mossy fibers and their expansions, *J. comp. Neurol.*, 195 (1981) 51-86.
- 4 Andersen, P., Organization of hippocampal neurons and their interconnections. In R. L. Isaacson and K. H. Pribram (Eds.), *The Hippocampus*, Plenum, New York, 1975, pp. 155-175.
- 5 Andersen, P., Holmqvist, B. and Voorhoeve, P. E., Entorhinal activation of dentate granule cells, *Acta physiol. scand.*, 66 (1966) 448-460.
- 6 Bakay, R. A. E. and Harris, A. B., Neurotransmitter, receptor and biochemical changes in monkey cortical epileptic foci, *Brain Research*, 206 (1981) 387-404.
- 7 Barber, R. and Saito, K., Light microscopic visualization of GAD and GABA-T in immunocytochemical preparations of rodent CNS. In E. Roberts, T. N. Chase and D. B. Tower, (Eds.), *GABA in Nervous System Function*, Raven, New York, 1976, pp. 113-132.
- 8 Curtis, D. R., Felix, D. and McLennan, H., GABA and hippocampal inhibition, *Brit. J. Pharmacol.*, 40 (1970) 881-883.
- 9 Falconer, M. A., The pathological substrate of temporal lobe epilepsy, *Guy's Hosp. Rep.*, 119 (1970) 47-60.
- 10 Fröscher, M., Misgeld, U. and Nitsch, C., Ultrastructure of mossy fiber endings in vitro hippocampal slices, *Exp. Brain Res.*, 41 (1981) 247-255.
- 11 Goldowitz, D., Vincent, S. R., Wu, J.-Y. and Hökfelt, T., Immunohistochemical demonstration of plasticity in GABA neurons of the adult rat dentate gyrus, *Brain Research*, 238 (1982) 413-420.
- 12 Green, J. D., The hippocampus, *Physiol. Rev.*, 44 (1964) 561-608.
- 13 Hsu, S.-M., Raine, L. and Fanger, H., Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures, *J. Histochem. Cytochem.*, 29 (1981) 577-580.
- 14 Königsmark, B. W., Methods for the counting of neurons. In W. J. H. Nauta and S. O. E. Ebbesson (Eds.), *Contemporary Research Methods in Neuroanatomy*, Springer, New York, 1970, pp. 315-340.
- 15 Lloyd, K. G., Munari, C., Bossi, L., Stoeffels, C., Talairach, J. and Morselli, P. L., Biochemical evidence for the alterations of GABA-mediated synaptic transmission in pathological brain tissue (stereo EEG or morphological definition) from epileptic patients. In P. L. Morselli, K. G. Lloyd, W. Löscher, B. Meldrum and E. H. Reynolds (Eds.), *Neurotransmitters, Seizures and Epilepsy*, Raven, New York, 1981, pp. 325-334.
- 16 Loskota, W. J. and Lomax, P., The Mongolian gerbil (*Meriones unguiculatus*) as a model for the study of the epilepsies: EEG records of seizures, *Electroencephalogr. clin. Neurophysiol.*, 38 (1975) 597-604.
- 17 Loskota, W. J., Lomax, P. and Rich, S. T., The gerbil as a model for the study of the epilepsies, *Epilepsia*, 15 (1974) 109-119.
- 18 Majkowski, J. and Bonadio, M., Electro-clinical studies of epileptic seizure in Mongolian gerbils, *Electroencephalogr. clin. Neurophysiol.*, 57 (1984) 369-377.
- 19 Nitsch, C. and Rinne, U., Large dense-core vesicle exocytosis and membrane recycling in the mossy fibre synapses of the rabbit hippocampus during epileptiform seizures, *J. Neurocytol.*, 57 (1981) 201-219.
- 20 Oertel, W. H., Schmechel, D. E., Weise, V. K., Ranson, D. H., Tappaz, M. L., Krutzsch, H. C. and Kopin, I. J., Comparison of cysteine sulphonic acid decarboxylase isoen-

- zymes and glutamic acid decarboxylase in rat liver and brain, *Neuroscience*, 6 (1981) 2701–2714.
- 21 Oertel, W. H., Schmechel, D. E., Mugnaini, E., Tappaz, M. L. and Kopin, I. J., Immunocytochemical localization of glutamate decarboxylase in rat cerebellum with a new anti-serum, *Neuroscience*, 6 (1981) 2715–2735.
  - 22 Ordonneau, P., Lindstrom, P. B.-M. and Petrusz, P., Four unlabeled antibody bridge techniques: a comparison, *J. Histochem. Cytochem.*, 29 (1981) 1397–1404.
  - 23 Paul, L. A., Fried, I., Watanabe, K., Forsythe, A. B. and Scheibel, A. B., Structural correlates of seizure behavior in the Mongolian gerbil, *Science*, 213 (1981) 924–926.
  - 24 Penfield, W., Epileptogenic lesions, *Acta neurol. belg.*, 56 (1956) 75–88.
  - 25 Ribak, C. E., Axon terminals of GABAergic chandelier cells are lost at epileptic foci, *Brain Research*, 326 (1985) 251–260.
  - 26 Ribak, C. E., Bradburne, R. M. and Harris, A. B., A preferential loss of GABAergic, symmetric synapses in epileptic foci: a quantitative ultrastructural analysis of monkey neocortex, *J. Neurosci.*, 2 (1982) 1725–1735.
  - 27 Ribak, C. E., Harris, A. B., Vaughn, J. E. and Roberts, E., Inhibitory GABAergic nerve terminals decrease at sites of focal epilepsy, *Science*, 205 (1979) 211–214.
  - 28 Ribak, C. E. and Seress, L., Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study, *J. Neurocytol.*, 12 (1983) 577–597.
  - 29 Ribak, C. E., Vaughn, J. E. and Saito, K., Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport, *Brain Research*, 140 (1978) 315–332.
  - 30 Seress, L. and Ribak, C. E., GABAergic cells in the dentate gyrus appear to be local circuit and projection neurons, *Exp. Brain Res.*, 50 (1983) 173–182.